

## Efficacy of Recombinant Methionyl Human Leptin Therapy for the Extreme Insulin Resistance of the Rabson-Mendenhall Syndrome

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Recombinant methionyl human leptin (r-metHuLeptin) therapy has shown clear efficacy in the treatment of severe insulin resistance associated with lipodystrophy syndromes and low leptin levels. We treated two siblings with Rabson-Mendenhall syndrome (severe insulin resistance and presumed insulin receptor mutations). The brother and sister, aged 13 and 11 yr, respectively, had severe acanthosis nigricans, insulin resistance, and diabetes. Both were taking 2000 mg metformin and 2 mg rosiglitazone daily; the brother was also taking 300 U regular insulin daily. In contrast to our lipodystrophic patients treated with r-metHuLeptin, these two patients had a higher percent body fat

and low-normal fasting triglycerides [42 mg/dl (0.37 mmol/liter), male sibling, and 33 mg/dl (0.47 mmol/liter), female sibling]. The siblings were treated with r-metHuLeptin therapy for 10 months and demonstrated a 40–60% decrease in fasting serum glucose and insulin levels and improved glycosylated hemoglobin. There was corresponding improvement in glucose and insulin tolerance during leptin therapy. This is the first report of a partial, but significant, effect of r-metHuLeptin administration in patients with extreme insulin resistance with a presumed insulin receptor mutation and low serum triglyceride levels. (*J Clin Endocrinol Metab* 89: 1548–1554, 2004)

THE RABSON-MENDENHALL (RM) syndrome is one of the most severe forms of insulin resistance, usually associated with marked decrease in insulin binding to its cell surface receptor caused by a mutation in the insulin receptor gene (1–4). The syndrome is characterized by marked insulin resistance, hyperinsulinemia, acanthosis nigricans, growth retardation, and various other phenotypic manifestations. Children initially have postprandial hyperglycemia, but eventually develop constant hyperglycemia from a progressive decline of endogenous insulin secretion.

The administration of recombinant methionyl human leptin (r-metHuLeptin) has been shown to improve insulin-stimulated hepatic and peripheral glucose metabolism in severely insulin-resistant lipodystrophic patients (5, 6). The mechanism by which leptin improves insulin sensitivity in lipodystrophic patients is unclear. The improvement has been correlated with the decrease in triglyceride content that occurs in serum, liver, and muscle during leptin therapy, but it is unclear whether this completely accounts for the increase in insulin sensitivity (6). Unlike lipodystrophic patients, patients with RM syndrome do not exhibit steatosis or hypertriglyceridemia. Treatment options are very limited in these patients, and their prognosis is poor (7). To determine whether leptin treatment might improve insulin sensitivity in a nonlipodystrophic condition, we studied two siblings

with RM syndrome. The two siblings also had relatively low leptin levels, diabetes mellitus, and acanthosis nigricans.

### Subjects and Methods

#### Patients

Two siblings with RM syndrome were studied. Both had histories of hypoglycemia when younger, growth retardation, multiple episodes of otitis media, and speech delay. By age 3 yr for the female and 4 yr for the male, they had been found to have acanthosis nigricans. The female was diagnosed with hyperinsulinemia at age 7 yr, and the male was found to be hyperinsulinemic as early as age 4 yr. Diabetes was first recognized at age 7 yr for the female and 9 yr for the male. The female was taking thiazolidinediones since age 8 yr, and the male since age 9 yr. Metformin was added to the treatment regimen at age 8 yr for the female and age 10 yr for the male. Insulin was added to the brother's treatment when he was age 12 yr.

Both children have fasting hyperglycemia, fasting hyperinsulinemia, severe glucose intolerance, abnormal dentition, and severe acanthosis nigricans, and hirsutism is present in the female (Fig. 1). Although insulin resistance is clear in these two siblings, they have not been studied by genetic analysis, and the likelihood that they have a mutation in their insulin receptor is presumed by their similarity to previously studied patients (1–4). Although in the past our laboratory has studied insulin receptor mutations, this technology is not available in the laboratory at present.

#### Study design

The study was designed as a 1-yr pilot study of two siblings. Data were collected at the Clinical Center of the NIH, Amgen, Inc. (Thousand Oaks, CA), provided recombinant methionyl human leptin. Data were held and analyzed by the NIH investigators. The study was approved by the institutional review board of NIDDK, and written informed assent and consent were obtained from the siblings and their parents. The response of each sibling was compared with his/her baseline values. The siblings were evaluated as inpatients before treatment, and again

Abbreviations: RM, Rabson-Mendenhall; r-metHuLeptin, recombinant methionyl human leptin.

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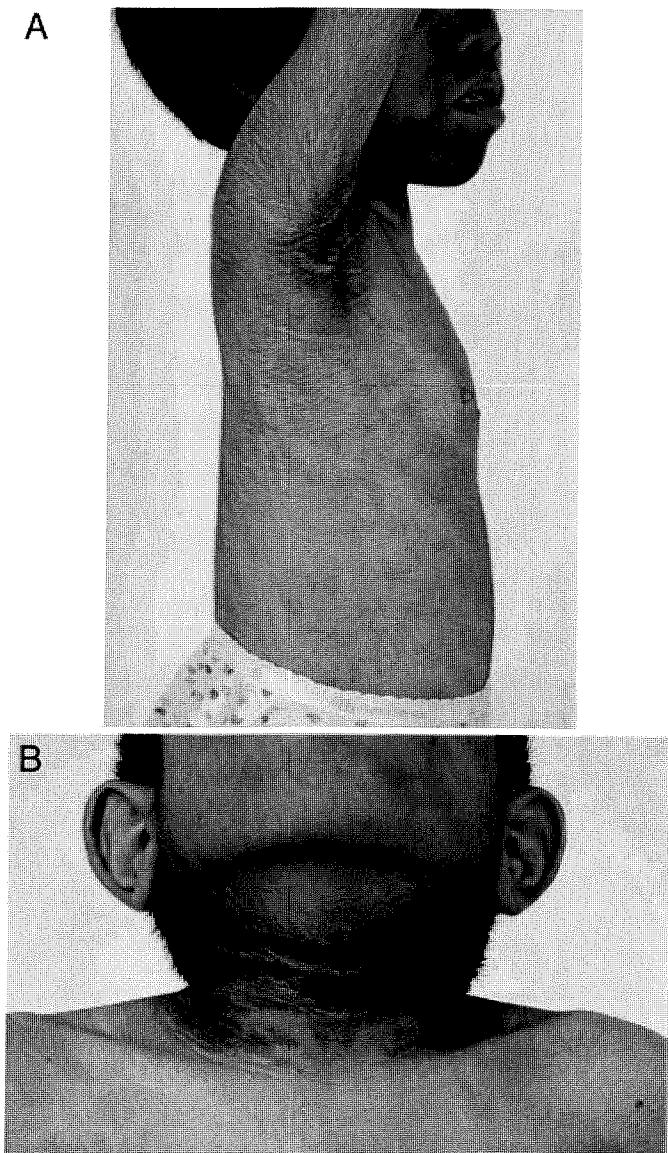


FIG. 1. Both children have fasting hyperglycemia, fasting hyperinsulinemia, severe glucose intolerance, abnormal dentition, and severe acanthosis nigricans; hirsutism is present in the female.

during 1, 2, 4, 6, and 10 months of r-metHuLeptin therapy. The patients were studied again 3 months after r-metHuLeptin therapy was withdrawn. The siblings had been receiving stable doses of other medications for at least 6 months before they began r-metHuLeptin administration. Both received metformin (500 mg, four times daily) and rosiglitazone (2 mg daily). In addition the male sibling received 300 U insulin daily. After 2 months on the study, the dose of insulin was decreased for the male sibling to 200 U insulin daily.

The r-metHuLeptin was administered sc every 12 h. The initial dose schedule was the same as that used in the lipodystrophy patients treated with r-metHuLeptin (5) (*i.e.* 0.03 mg/kg-d for the female and 0.02 mg/kg-d for the male). The siblings were treated with 50% of the initial dose for the first month, 100% for the second month, and 200% from the third month through the sixth month. From the sixth month through the 10th month of therapy, the siblings were treated with 300% of the prescribed initial dose (*i.e.* 0.09 mg/kg-d for the female and 0.06 mg/kg-d for the male). The r-metHuLeptin therapy was stopped after 10 months of treatment, and metabolic parameters were obtained 3 months after withdrawal for both siblings.

### Biochemical analyses

All samples for analyses were collected in the morning after an overnight fast. Serum glucose and triglyceride levels were determined according to standard methods with the use of automated equipment (Hitachi, Roche, Indianapolis, IN). Glycosylated hemoglobin values were measured by ion exchange HPLC (Bio-Rad Laboratories, Hercules, CA). Serum insulin levels were determined by immunoassays with the use of reagents provided by Abbott Instruments (Chicago, IL). Serum leptin levels were determined by immunoassays with the use of a commercial kit (Linco Research, Inc., St. Charles, MO).

Serum FSH and LH concentrations were assayed using a microparticle enzyme immunoassay on an AxSYM system (Abbott Diagnostics). Serum TSH levels were measured with a two-site chemiluminescent immunometric assay on DPC Immulite 200 equipment (Diagnostic Products Corp., Los Angeles, CA). Serum T<sub>3</sub>, T<sub>4</sub>, and T<sub>4</sub>-binding globulin, and cortisol levels were also determined via competitive immunoassays, namely via chemiluminescent immunometric assay on DPC Immulite 200 equipment (Diagnostic Products Corp.). Free T<sub>4</sub> was measured with an electrochemiluminescent competitive immunoassay on Elecsys 2010 equipment (Roche). For rT<sub>3</sub> determination, an RIA developed by the Mayo Medical Laboratories (Rochester, MN) was used.

A combined TRH and GnRH test was performed after an overnight fast and before administration of any medication. Protirelin [7 µg/kg; maximum, 500 µg; Ferring Pharmaceuticals Ltd. (Tarrytown, NY) and Taylor Pharmaceuticals (Decatur, IL)] and gonadorelin (100 µg; Factrel, Ayerst Pharmaceuticals, Philadelphia, PA) were administered as iv boluses. Blood samples were drawn at -15, 0, 5, 15, 30, 60, 120, and 180 min for FSH, LH, and TSH determinations and at 0 and 180 min for T<sub>3</sub> and T<sub>4</sub> determinations.

### Procedures

During each visit the resting metabolic rate was measured by metabolic cart (Deltatrac equipment, Sensormedics, Yorba Linda, CA), between 0600–0800 h while patients rested after an overnight fast of more than 8 h. Each patient underwent a 3-h oral glucose tolerance test in which 1.75 g/kg dextrose oral solution (75 g/300 ml) were administered orally. A high dose insulin tolerance test was performed with the use of 0.2 U regular insulin/kg to assess the siblings' sensitivity to insulin.

Body fat was determined using a dual energy x-ray absorptiometer (model QDR 4500, Hologic, Bedford, MA) (8). Axial T<sub>1</sub>-weighted magnetic resonance imaging of the liver was performed with the use of a 1.5-T scanner (General Electric Medical Systems, Milwaukee, WI). Liver volumes were calculated with the use of the MEDx image analysis software package (Sensor Systems, Sterling, VA).

Before their baseline and 4 month admissions, subjects, with the assistance of their parents, kept 5- to 7-d food records. These records were reviewed for accuracy and completeness with the children and their mother by a research dietitian. Nutrient calculations were performed using the Nutrition Data System for Research software versions 4.03 and 4.04, developed by the Nutrition Coordinating Center, University of Minnesota (Minneapolis, MN; Food and Nutrient Databases 31 and 32) (9).

## Results

### Baseline characteristics

Both siblings demonstrated the phenotypic features of RM syndrome, with severe hirsutism and acanthosis nigricans (Fig. 1), and the characteristic growth retardation of this syndrome (Fig. 2). Serum leptin levels were appropriate for fat mass. Patients had low normal serum triglyceride levels and no evidence of hepatic enlargement. They were diabetic and had marked hyperinsulinemia (Table 1).

### Response of metabolic parameters to leptin therapy

The r-metHuLeptin was administered in gradually increasing doses that remained stable from the sixth to the 10th month. After 10 months of r-metHuLeptin therapy, the overnight fasting leptin concentration increased from 4.5–8.3

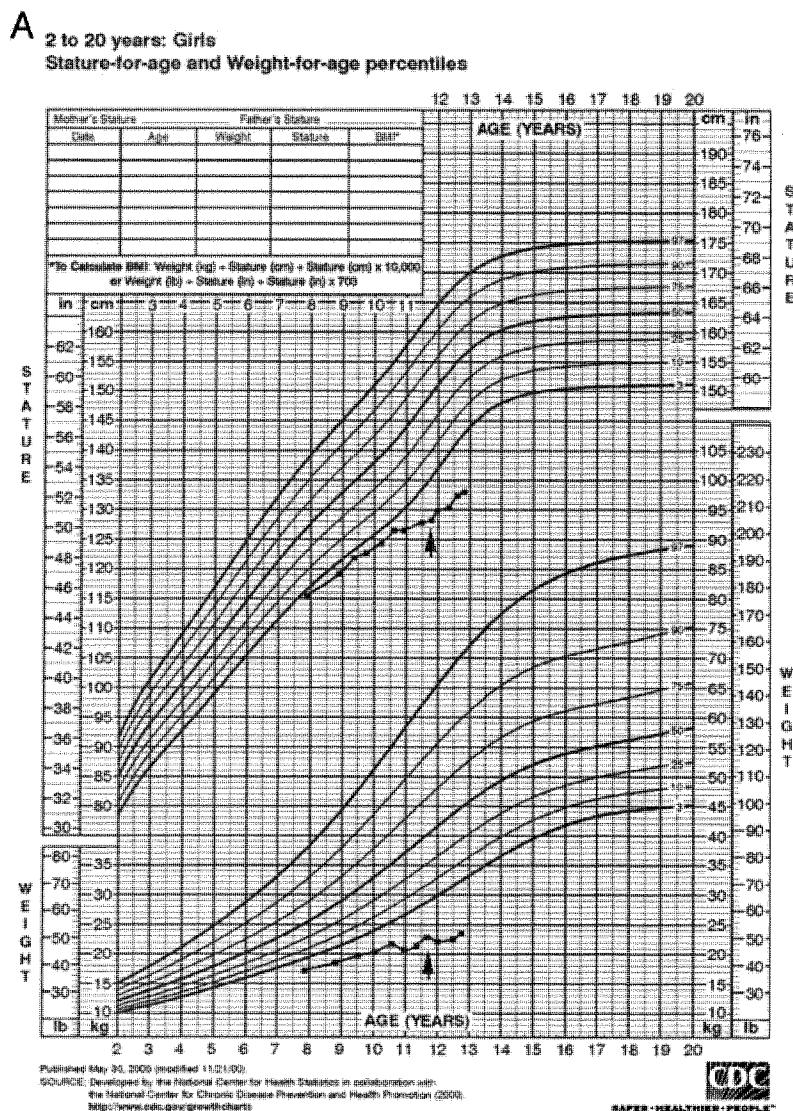


FIG. 2. Growth charts for the female (A) and male (B) siblings. The characteristic growth retardation of the RM syndrome is shown. The arrow indicates when leptin therapy began.

TABLE 1. Baseline metabolic parameters

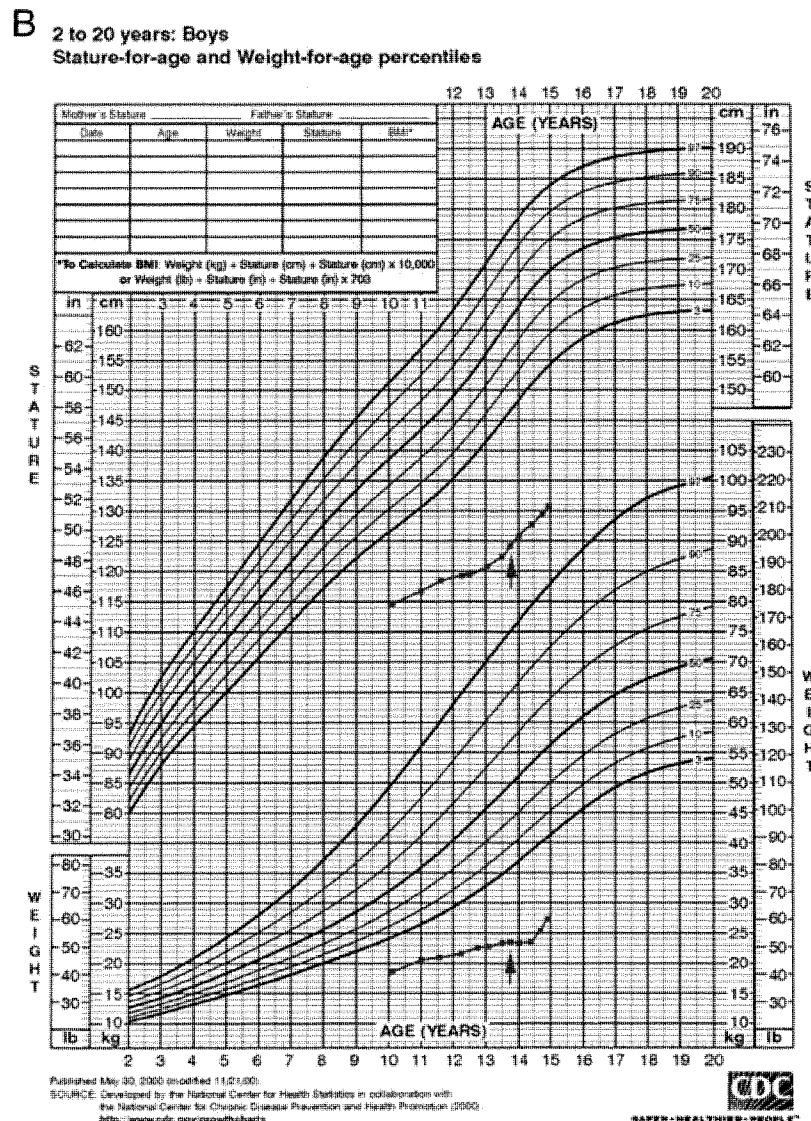
	Age (yr)	Height (cm)	Weight (kg)	Leptin (ng/ml)	Insulin ( $\mu$ U/ml)	Glucose (mg/dl)	TG (mg/dl)	Hyperglycemic therapy
Female	11	129	22	4.5	194	153	33	Metformin (200 mg QD) Rosiglitazone (2 mg QD)
Male	13	124	23	3.4	268	224	42	Metformin (2000 mg QD) Rosiglitazone (2 mg QD) Insulin (300 U QD)

To convert to SI units: insulin, multiply by 6.945 to obtain picomoles per liter; glucose, multiply by 0.0555 to obtain millimoles per liter; triglycerides (TG), multiply by 0.0113 to obtain millimoles per liter. QD, Daily.

ng/ml in the female subject and from 3.4–5.7 ng/ml in the male; fasting blood glucose fell by 40% in the female and by 60% in the male from their baseline levels. This improvement in fasting hyperglycemia in the male occurred despite reducing his daily dose of insulin by one third, from 300–200 U/d. Both children exhibited an overall reduction of percent glycosylated hemoglobin and a reduction of fasting insulin levels. The fasting insulin level decreased by 80% in the female patient, who was not receiving exoge-

nous insulin. All of these values returned to or exceeded the original baseline values after r-methHuLeptin withdrawal (Table 2).

A 1.75 g/kg oral glucose tolerance test demonstrated the basal glucose levels of both patients to be decreased after 10 months of therapy, but both patients still had abnormal glucose tolerance. Further insulin values were decreased at all time points in the female patient after 10 months of therapy (Fig. 3). During a 30-min insulin tolerance test, the average

FIG. 2. *Continued***TABLE 2.** Fasting metabolic changes during leptin therapy

	Female patient			Male patient		
	Preleptin therapy	During therapy	3 months off therapy	Preleptin therapy	During therapy	3 months off therapy
Leptin (ng/ml)	4.5	8.3	4.3	3.4	5.7	3.4
Insulin (μU/ml)	194	39	289	268 <sup>a</sup>	49 <sup>b</sup>	168 <sup>b</sup>
Glucose (mg/dl)	153	91	204	231	92	221
Triglyceride (mg/dl)	33	67	71	42	105	68
HgbA <sub>1c</sub> (%)	9.2	8.3	8.9	9.6	8.3	10

To convert to SI units: insulin, multiply by 6.945 to obtain picomoles per liter; glucose, multiply by 0.0555 to obtain millimoles per liter; triglyceride, multiply by 0.0113 to obtain millimoles per liter. HgbA<sub>1c</sub>, Glycosylated hemoglobin.

<sup>a</sup> The male patient is taking 300 U insulin per day.

<sup>b</sup> The male patient is taking 200 U insulin per day.

reduction in serum glucose was approximately 40% after the injection of 0.2 U/kg insulin in the two children compared with their baseline data after 10 months of r-metHuLeptin therapy. Again, the values for glucose during the insulin tolerance test returned to or exceeded the original baseline after leptin withdrawal.

#### Growth and development after leptin therapy

At baseline both children had marked growth retardation, which is characteristic of this syndrome (Fig. 2). They both continued to grow at a rate below the third percentile after leptin administration. There was no loss of weight or lean

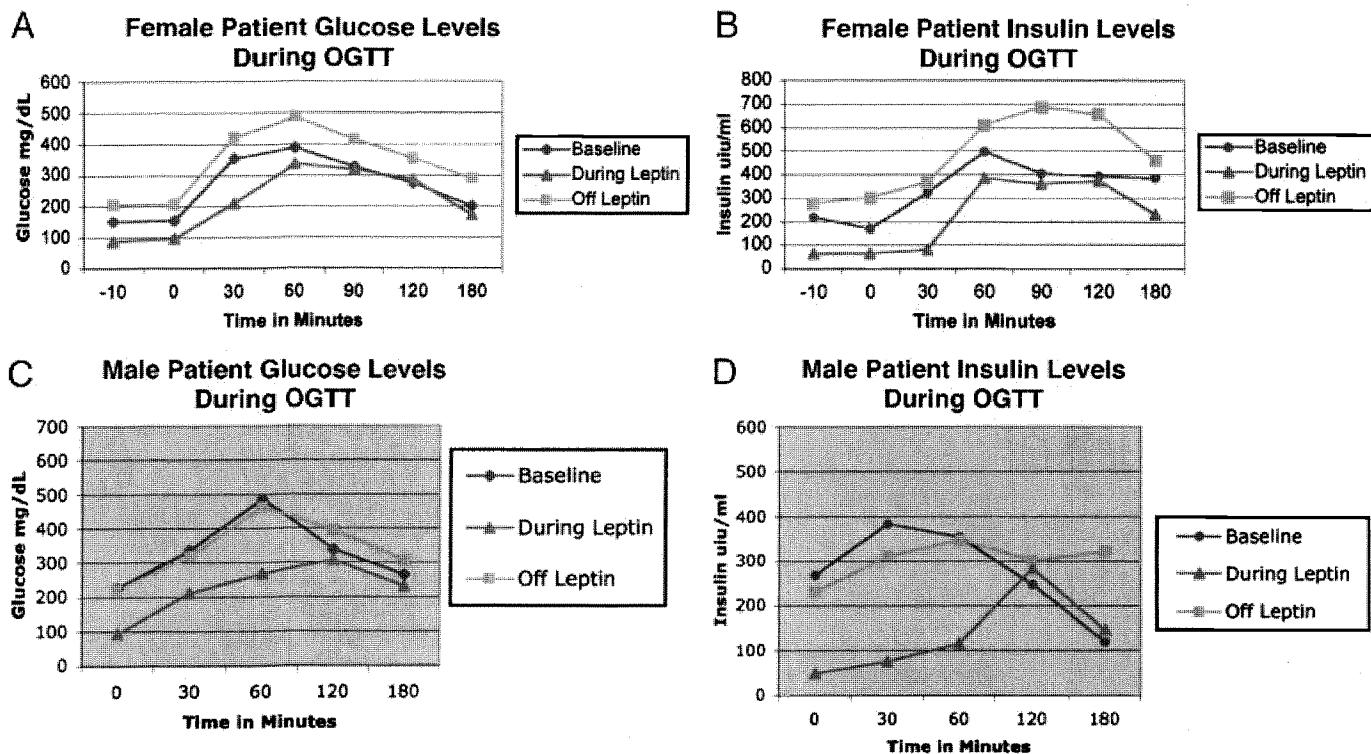


FIG. 3. A and C, Glucose levels during the oral glucose tolerance test (OGTT) in the female (A) and male (C) siblings. B and D, Insulin levels during the OGTT in the female (B) and male (D) siblings.

TABLE 3. Body composition during leptin therapy

	Female patient			Male patient		
	Preleptin therapy	During therapy	3 months off therapy	Preleptin therapy	During therapy	3 months off therapy
Height (cm)	128	130	133	124	126	131
Weight (kg)	22	21.5	23	23	23	27
Fat g (subtotal)	3382	2719	3704	3527	2181	2738
Lean g (subtotal)	15619	15748	16234	16503	17241	20225
Respiratory quotient	0.8	0.83	0.78	0.8	0.8	0.79
REE (kcal/24 h)	1100	1000	910	1019	1030	1200
% Fat (subtotal)	17.4	14.4	18.2	17.3	11.0	11.7

REE, Resting energy expenditure.

body mass after leptin therapy, but there was a decrease in percent body fat during leptin therapy (Table 3). The average caloric intake for each sibling decreased approximately 200 kcal/d during therapy, from 1326 to 1227 kcal in the male sibling and from 1718 to 1528 kcal in the female sibling. Consistent with lean body mass, resting energy expenditure remained stable during leptin therapy (Table 3).

#### Hypothalamic-pituitary function

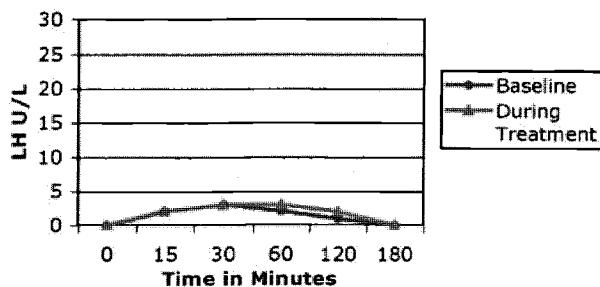
At baseline the male subject had a normal response to GnRH, consistent with normal pubertal development, whereas the younger female sibling had no response to GnRH at baseline or at 4 months of leptin therapy (Fig. 4). This is consistent with the theory that leptin is permissive for these responses when puberty is already in progress, but that leptin does not induce puberty *per se*. Thyroid and adrenal function were normal and unaffected by leptin therapy (data not shown).

#### Discussion

In the present study we have shown that pharmacological doses of leptin administered to two siblings with RM syndrome results in improvement of fasting hyperglycemia, hyperinsulinemia, glucose, and insulin tolerance. These children had normal baseline leptin concentrations for their percent body fat. We interpret these clinical changes as being consistent with decreased insulin resistance. The metabolic improvements are similar to improvements that have been seen in leptin-deficient, lipodystrophic patients (5). In these lipodystrophic patients, the clinical improvement has corresponded to increased insulin sensitivity measured by the euglycemic clamp (6).

Several caveats of the study are important to note. Although the patients had extreme insulin resistance and were similar to other patients who have been proven to have mutations in the insulin receptor (1–4, 7), molecular genetic analyses of these two patients have not been performed.

### A LH Levels During LHRH Test Female Patient



### B LH Levels During LHRH Test Male Patient

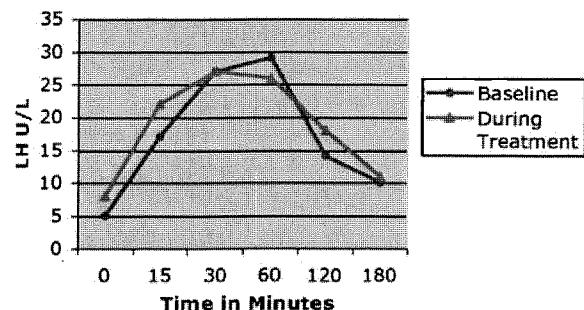


FIG. 4. LH levels during the LH-releasing hormone (LHRH) test.

Further, the effect of r-metHuLeptin therapy in these patients is partial with respect to glucose metabolism. Whether escalation of the leptin dose will give a greater effect has not been determined.

The improvement in glucose and insulin parameters shown here is similar to that seen in lipodystrophic patients with one major difference. In lipodystrophy, hypertriglyceridemia is a major abnormality, and reduction in triglycerides is one of the major effects of leptin therapy. In these two siblings, triglyceride levels were normal or low, and there was no effect of r-metHuLeptin therapy.

Several other comparisons are of interest. In contrast to our adolescent and adult lipodystrophic patients, who have achieved their expected adult height, these siblings had no loss of weight with r-metHuLeptin therapy and no loss of lean body mass (10, 11). Thus, they are similar to growing children with congenital leptin deficiency, in whom there is no loss of lean body mass, but a major loss of fat mass during r-metHuLeptin administration (10, 11). Our two sibling patients, who are lean compared with children with congenital leptin deficiency, also showed a modest loss of fat mass during r-metHuLeptin therapy. A reduction in caloric intake was observed (~11%) in the sibling patients. This is less than what was observed in our lipodystrophic patients, in whom, on the average, there was a 45% reduction in caloric intake (11).

Similar to our previous observations (12), leptin appears permissive for gonadotropin secretion once puberty occurs, but leptin does not induce puberty *per se*, in humans, nor does

r-metHuLeptin therapy have any effect on thyroid or adrenal function as seen in rodents (13).

The mechanism by which leptin improves insulin sensitivity in our patients is unclear. In rodent models several mechanisms could be relevant. This includes the activation of 5'-AMP kinase, which will increase insulin sensitivity (14). Further, it is possible that there could be an effect on an insulin receptor substrate, such as insulin receptor substrate-2, that could be affected by leptin therapy directly or indirectly by decreasing endogenous hyperinsulinemia (15). As there is no effect on serum lipids in these patients, it is unlikely that the stearoyl desaturase pathway is being influenced by leptin therapy as in lipodystrophy (16). Unlike lipodystrophic human syndromes, which have excellent rodent models, there are no clear rodent models for the RM syndrome (17).

Longo *et al.* (18) report a progressive decline in insulin levels in the RM syndrome with increasing age of the patient. This progressive decline in insulin levels leads to worsening diabetes and diabetic ketoacidosis in these patients. During the 10 months of leptin therapy it has been shown that the two subjects had a progressive decline in their insulin levels. However, these insulin levels increased to preleptin therapy levels or higher when checked at 3 months off treatment for the female and the male patient.

Most importantly, at the clinical level, these children were taking significant amounts of insulin-sensitizing medications at baseline; the male child was taking at least 13 times the usual replacement dose of insulin. Against this background, leptin therapy has produced the most significant clinical improvement that we have seen in these extremely insulin-resistant children. Whether leptin therapy will improve the grim prognosis for these children remains to be seen.

### Acknowledgments

Received November 11, 2003. Accepted January 15, 2004.

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